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ABSTRACT

This report summarizes investigations conducted by the Life Sciences Department of the Space and Information Systems Division (S&ID) of North American Aviation, Inc., under the Contract NAS2-1539, Biosatellite Experiment - P 1017, "Study of the Liminal Angle of a Plagiogeotropic Organ Under Weightlessness." Included is a discussion of the general background and requirements underlying the study effort. Specific procedures and results are summarized and discussed. In addition, the requirements, constraints, design features, and test results of laboratory preprototypes are presented. (Preprototypes were designed and fabricated to serve as a basis for flight hardware.) The general requirements and design considerations for a spaceflight biological package are described. Finally, overall study findings are summarized, with specific problem areas and recommendations for further research.

(5) North American Aviation Inc., Downey, Calif.

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FINAL REPORT FOR STUDY OF THE LIMINAL ANGLE OF A PLAGIOGEOTROPIC ORGAN UNDER WEIGHTLESSNESS

Contract NAS2-1539

G.O. 5153

29 March 1964

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SPACE and INFORMATION SYSTEMS DIVISION

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ABSTRACT

This report summarizes investigations conducted by the Life Sciences Department of the Space and Information Systems Division (S&ID) of North American Aviation, Inc., under the Contract NAS2-1539, Biosatellite ~~Experiment~~ - P-1017, Study of the Liminal Angle of a Plagiogeotropic Organ Under Weightlessness. Included is a discussion of the general background and requirements underlying the study effort. Specific procedures and results are summarized and discussed. In addition, the requirements, constraints, design features, and test results of laboratory preprototypes are presented. (Preprototypes were designed and fabricated to serve as a basis for flight hardware.) The general requirements and design considerations for a spaceflight biological package are described. Finally, overall study findings are summarized, with specific problem areas and recommendations for further research.

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INTRODUCTION

STUDY BACKGROUND AND REQUIREMENTS

It has long been established that higher plants subjected to horizontal rotation along the main axis (stem axis) will respond consistently to applied omnilateral geotropic stimulation. One result of such stimulation is a marked reduction of the liminal angle of the petioles (leaf stalks). The liminal angle is defined as the angle between the ventral surface of the petiole and the plant stem. The clinostat (Figure 1) is an instrument employed to provide horizontal rotation of higher plant forms. Finn and Brown[1] constructed a variable-speed 13-foot centrifuge (Figure 2), with eight radially oriented, horizontal clinostats equally distributed about its periphery. This apparatus was designed to accommodate the mounting of potted plants with either apical or root ends nearest the axis of the centrifuge. With this centrifuge-horizontal clinostat apparatus, a series of studies were conducted using Xanthium pensylvanicum as the plant species. It was found that reduction in the liminal angle of Xanthium due to horizontal clinostat rotation was reversed when simultaneous apex-to-root centrifugation of 1-g equivalent was applied. Subsequent accelerations between 0- and 1-g were applied, utilizing the centrifuge-clinostat device. Results of the studies indicated that a smooth, reproducible curve of changes in liminal angle can be plotted as a function of acceleration. The petiole positions itself in opposition to the resultant forces of the mass acceleration field when the acceleration is applied apex to root.

The results of these efforts have indicated that horizontal clinostat rotation may be of potential value as a simulation technique for studying plant behavior in modified gravitational fields. It remains necessary to establish that the effect of rotation on a horizontal clinostat is equivalent to placing a plant in a zero mass-acceleration field. If equivalency is established, the response of higher plants to subgravity fields (e.g., 1/6 g, as present on the lunar surface) may be studied by economical earth-based techniques. In addition, the centrifuge-clinostat technique would permit extension of inquiry into the basic processes involved in georeactions.

PURPOSE OF THE STUDY EFFORT

The basic working hypothesis of the investigation was that the marked liminal-angle reduction resulting from rotation on a horizontal clinostat is equivalent to the behavior of petioles in a weightless state. Verification of the hypothesis can be provided solely by actual in-space biological experimentation.

[1]Members of S&ID Life Sciences Department

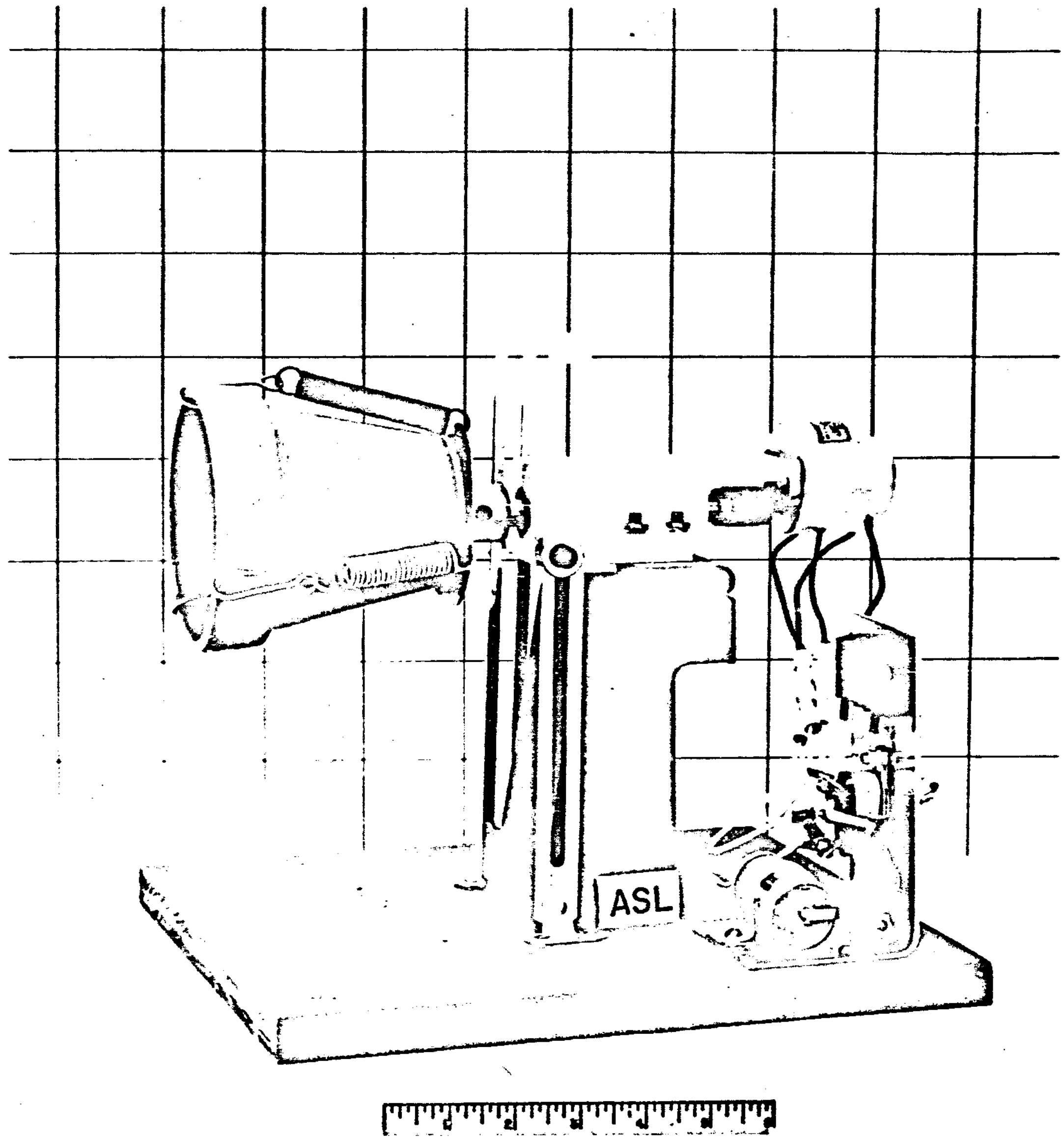

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Figure 1. Adjustable Clinostat

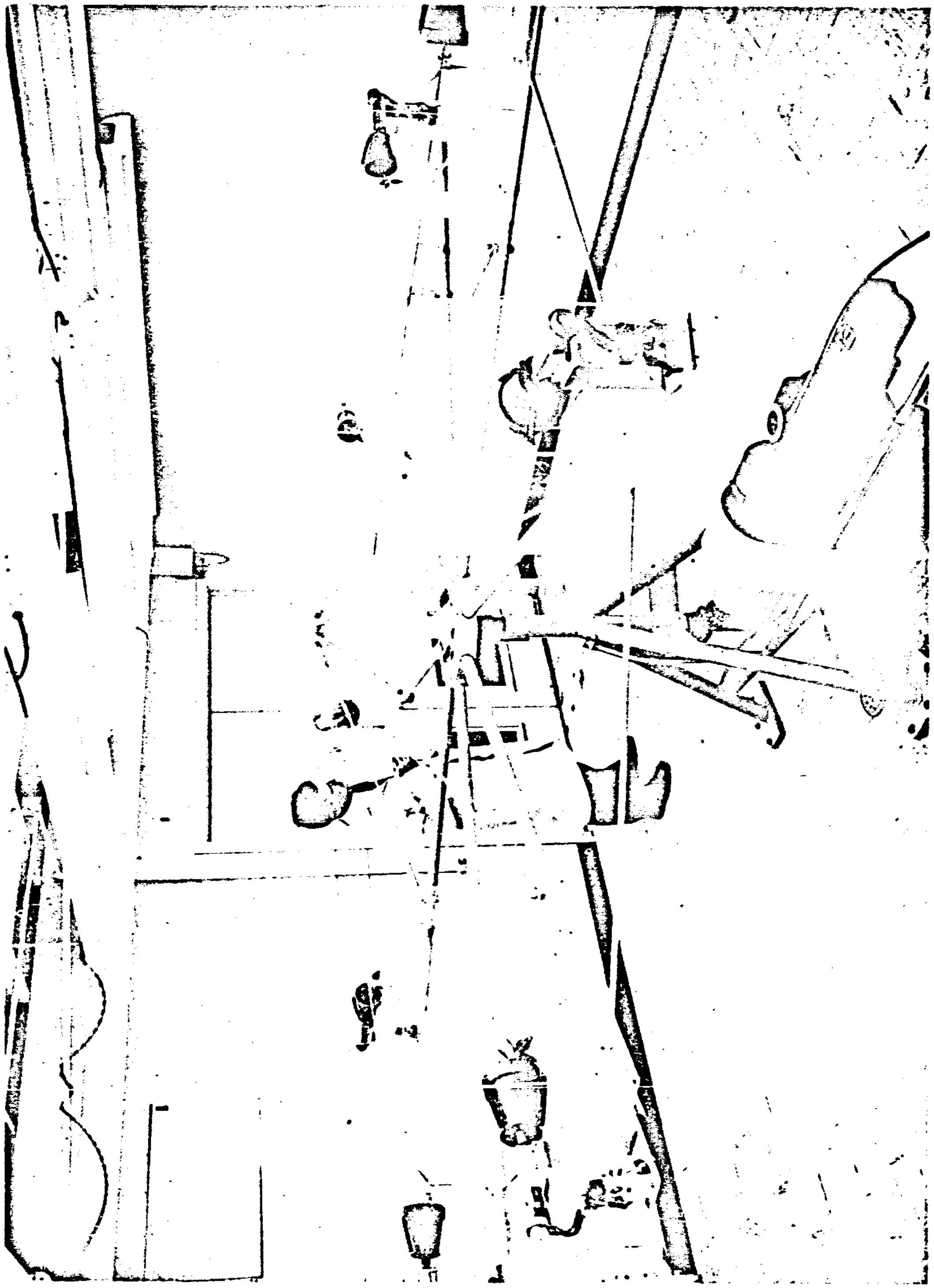

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Figure 2. Clinostat-Centrifuge Apparatus

EXPERIMENTAL PROGRAM

APPROACH

In order to provide sufficient background data for the experiment to test the hypothesis, investigations were classified into four major categories:

1. Experiments to define plant responses under various environmental conditions

2. Experiments to provide constraints and conditions required in defining a flight package

3. The design and fabrication of preprototype hardware

4. Studies to elaborate areas of research interest to increase the value of the biological experiment

INTRODUCTORY LABORATORY INVESTIGATIONS

Selection of Plant Species

Several higher forms were assessed for potential suitability in the spaceflight package. Criteria for selection involved consideration of:

1. Compact plant size

2. Small leaf blades

3. Sturdy petioles

4. Marked changes in liminal angle during experimental procedures.

5. Ability of plant to withstand anticipated rigors of the flight.

The plant Xanthium pensylvanicum utilized in prior investigations was rejected because large-sized seedlings were required to exhibit responsiveness. Holly, oleander, and olive plants (6.5 to 8.5 cm in height) possessed durable petioles but failed to exhibit appropriate plagiogeotropic response. Coleus was considered but not selected because of its delicate nature and tendency to wilt readily. The tomato plant was rejected because of its extreme response to horizontal rotation. Capsicum annuum (Yolo Wonder

Bell Pepper) exhibited a marked reduction of the liminal angle when subjected to horizontal clinostat rotation. As a result of preliminary testing, Capsicum annuum was selected for use in further studies because it adequately met all criteria mentioned previously.

Plant Preparation and Care

Capsicum annuum seeds were planted in wooden flats, 45 x 45 x 8 cm, containing a substrate mixture consisting of sandy soil, vermiculite, and peat moss in equal parts. The soil mixture was used in most of the tests. Nutri-Foam (Dow Chemical Company) was employed in specific tests to evaluate the use of a synthetic organic substrate.

The seeds were not pre-soaked. After approximately two weeks, the seedling (approximately 3 cm high) were transplanted to ceramic pots, 10 cm in diameter, containing either the soil mixture or Nutri-Foam. The individual flats and pots were watered daily. A nutrient solution was applied to the seedlings in the soil mixture at weekly intervals beginning with the first week following planting. (See Appendix A for Nutrient Solution.) Plants positioned on the clinostats were watered either by injection of water into the substrate with a hypodermic needle, or by surface watering. Preventive control against insects and fungi was provided through the use of Rose and Flower Spray and Orthocide (California Chemical Company, Ortho Division).

In the standard culture condition, all plants were maintained at 27 ± 3 C with a continuous 400 footcandle illumination, provided by Powergroove fluorescent lamps (General Electric Company).

Laboratory Apparatus

Clinostats

The clinostats employed were described previously. An example is shown in Figure 1. The instruments employed in these studies were designed and constructed by North American Aviation, Inc. The motors rotated the potted plants at 1/6 rpm.

Centrifuge

The centrifuge employed was described previously and shown in Figure 2. The angular acceleration produced was calculated by one of the following formulae:

$$A = \omega^2 R \qquad (1)$$

where ω = radians/sec

R = radius in feet

$$\frac{C}{g} = \frac{4\pi^2 r}{gt^2} \qquad (2)$$

where

r = radius in meters

t = time in seconds for one revolution.

The force on the variable clinostats was calculated by selecting an angle up from the horizontal, the sine of which would yield the appropriate g-loading. Angles of 0, 14.5, 30, 48.6, and 90 degrees were used to achieve g-loadings of 0, 0.25, 0.50, 0.75, and 1.0-g respectively.

Device for Estimating Liminal Angle

The liminal angle was measured with a transparent protractor, equipped with a movable marker. In practice, the complement, β, of the liminal angle, α, is determined by placing the protractor in the position shown in Figure 3. The tangent to a circle at the point where the petiole becomes confluent with the leaf blade is estimated. The liminal angle, α, may then be calculated by subtracting the complementary angle, β, from 180 degrees. The stem apex is considered the zero reference point.

Determination of Minimum Plant Size

Space and volume limitation expected in the biological satellite imposed the requirement for determining the minimum size plant which would adequately exhibit petiole response. The criterion for adequacy of responses was a 50 degree minimum reduction in the liminal angle of four (4) petioles within 24 hours. The decision to evaluate four (4) petioles was based upon the production of a reasonable number of data points. A greater number of leaves was not considered because of difficulties anticipated in direct reading and in a photographic interpretation. For purposes of identification in these studies, the first four leaves to appear (excluding the cotyledons) were consistently labeled A, B, C, and D, in order of their appearance. Plants of various ages were rotated on horizontal clinostats for 24 hours. While still rotating, they were subsequently exposed to a calculated 1 g by one of two methods, for an additional 24 hours. In Method A, the eight plants which were equally distributed about the periphery of the centrifuge received a calculated 1 g. In Method B, individual clinostats were set in the vertical position. Plants of the following ages and heights were employed in these tests:

1. Two weeks (average height 1.4 cm, range 0.4 to 2.7 cm)

2. Three weeks (average 1.9 cm, range 0.8 to 3.6 cm)

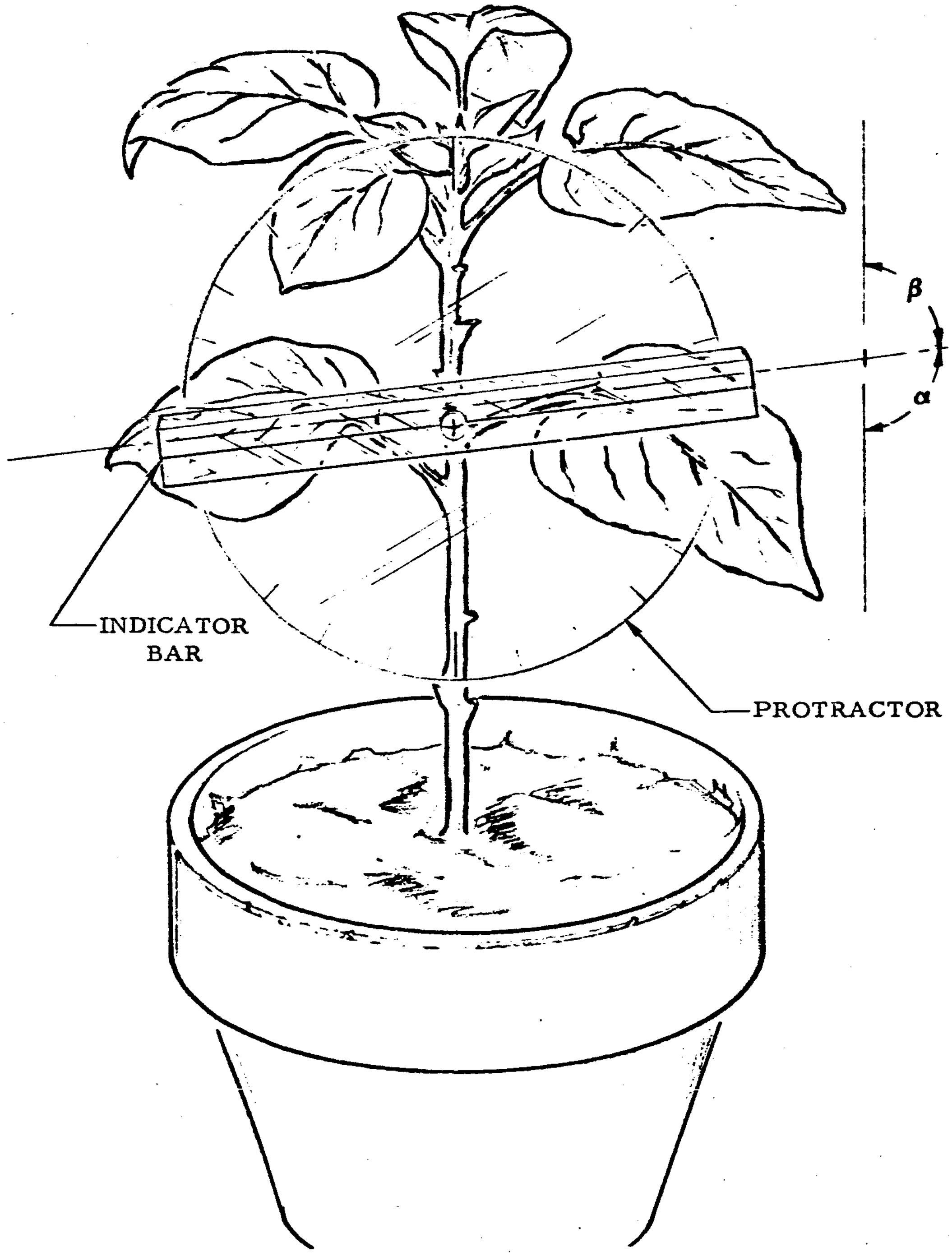

Figure 3. Method of Estimating Liminal Angle

3. Four weeks (average 3.9 cm, range 3.0 to 5.3 cm)

4. Six weeks (average 4.8 cm, range 3.5 to 6.5 cm).

Table 1 summarizes the average responses of the petioles of six-week-old plants. The responses of the two-, three-, and four-week-old plants are summarized in Appendix B. It appears that the age of the plant is more significant than the height for adequate petiolar response. For example, a four-week-old plant 4.8-cm tall showed less response for leaves C and D than did a six-week old plant 4.2-cm tall. Weight measurements were made following transplanting. Six-week-old plants showed a significant response in four leaves.

Table 1. Responses of Six-Week-Old Plants

Plants	Condition	Time from Start (hour)	Average Liminal Angle (degree) A	B	C	D
1 to 8	Horizontal	0	131	130	139	137
	Horizontal	24	73	73	52	58
	1-g*	48	112	117	142	140
9 to 16	Controls	0	129	133	134	136
		24	130	134	136	139
		49	133	136	138	137
17 to 24	Horizontal	0	129	135	133	138
	Horizontal	24	84	84	65	76
	1-g**	48	98	95	98	113

*g-loading applied by placing clinostat upright
**g-loading applied by centrifugation

DEFINITION OF PLANT RESPONSES

Continuous Observation of Plant Responses

Observation of the liminal angle was undertaken on a round-the-clock basis to achieve the following:

1. Increased number of data points

2. Detection of subtle changes, (e.g., superimposition of a rhythm)

3. Determination of the time required by the plants to equilibrate at selected modified g

4. Determination of approximate time interval for significant photographic coverage of plant response.

Readings of the liminal angles of 16 experimental plants were taken every two hours. The plants were exposed to g-load profiles of 0, 0.25, 0.50, 0.75, and 1.0.

The duration of exposure was the period of time needed to achieve equilibrium. Equilibrium in liminal-angle response was defined as the point in time at which there was no appreciable change in two consecutive readings.

The results of these tests indicated a significant variation in response time and angular movement among plants within any one group. The value of obtaining increased data during this run was overshadowed by the intrinsic variability in response displayed by individual plants. Subtle effects exhibited by the liminal angle and the exact time required before the observance of equilibrium were masked for the same reason. Prior to the observance of equilibrium, definite angular change was noted in all cases. It may be assumed, therefore, that photographic coverage of less than two-hour intervals will result in meaningful data. Selection of optimum interval between readings will be determined in later experiments.

Plant Selection by Prior Testing

The inherent variation in angular response observed in the above experiments dictated an investigation of methods to select plants which will respond in a uniform manner.

The conduct of valid in-flight experimentation is highly dependent upon the availablility of accurate baseline data on the particular plants selected for the flight package. Such baseline data is necessary for two reasons:

1. Data is required to insure the selection of homogeneous plant samples

2. Reference data is required as a basis for subsequent comparison of in-flight versus terrestrial results.

The assembly of baseline reference data for individual plants selected for in-flight experimentation requires that plants be thoroughly pretested to ascertain their response characteristics prior to flight. The question then arises as whether or not an individual plant can be pretested without altering its characteristic responses to subsequent modified g. In order to answer the above question, it was necessary to run a series of tests to determine the effect of repeated exposure of plants to modified g environments. If the results of these tests indicated no significant change in response potential or characteristics of exhibited response, the inference could be made that plants may be pretested for response prior to launch. Plants were rotated on horizontal clinostats for 24 hours and the changes in liminal angle were recorded. The plants were then removed from the clinostats and restored to their normal orientation for an additional 24 hours. The process was repeated with the plants two and three times. The two-cycle regimen was established to predict the response of the plants to a simulated zero-g flight after a ground test. The results are presented in Table 2.

Emphasis was placed upon the behavior of individual petioles during this phase of the investigation, anticipating that a trend could be established which would be valid for plant selection. In analyzing the data presented in Table 2, the following arbitrary criteria were established to demonstrate the predictive advantages of a prior testing procedure:

1. After the first 24-hour horizontal clinostat rotation, the liminal angle must be reduced by more than 50, but less than 100 degrees.

2. The liminal angle must then recover 95± 10 percent following 24 hours of normal orientation.

3. Petioles meeting these criteria must then exhibit at least a 50-degree alteration in liminal angle following a second 24-hour exposure to horizontal rotation.

Out of a total of 18 plants, it was observed that all eight D petioles which met the first two criteria also met the third. Of nine C petioles, which met Criteria 1 and 2, seven satisfied the third criterion. Only one B petiole met all three criteria; 3 B petioles met the first two. Of six A petioles fulfilling Criteria 1 and 2, five also fulfilled the third. The above data are summarized in Table 3.

Table 2. Evaluation of Repeated Exposure to Modified Gravity

Plants		Change in Liminal Angle 1st Exposure (degree)	Recovery (percent)	Change in Liminal Angle 2nd Exposure (degree)
1	A	70	93	45
	B	80	63	30
	C	20	25	25
	D	45	122	85
2	A	85	41	35
	B	65	54	50
	C	15	67	65
	D	90	89	70
3	A	65	46	30
	B	70	86	45
	C	70	86	75
	D	75	100	50
4	A	60	75	45
	B	65	69	35
	C	75	93	70
	D	60	100	65
5	A	50	100	65
	B	55	64	35
	C	75	87	60
	D	60	92	95
6	A	60	25	45
	B	60	33	55
	C	20	25	65
	D	35	157	80
7	A	50	90	50
	B	55	73	45
	C	40	113	75
	D	65	108	80

Table 2. Evaluation of Repeated Exposure to Modified Gravity (Cont.)

Plants		Change in Liminal Angle 1st Exposure (degree)	Recovery (percent)	Change in Liminal Angle 2nd Exposure (degree)
8	A	45	100	55
	B	55	82	50
	C	80	113	75
	D	105	105	75
9	A	85	82	15
	B	105	86	60
	C	70	100	105
	D	95	100	80
10	A	30	83	60
	B	15	200	75
	C	95	105	105
	D	70	136	75
11	A	40	100	55
	B	50	120	70
	C	55	118	60
	D	115	91	65
12	A	100	90	50
	B	90	83	40
	C	80	75	45
	D	75	107	60
13	A	45	56	25
	B	30	100	35
	C	65	92	65
	D	55	118	85
14	A	70	93	70
	B	40	113	65
	C	80	113	75
	D	65	115	85

Table 2. Evaluation of Repeated Exposure to Modified Gravity (Cont.)

Plants		Change in Liminal Angle 1st Exposure (degree)	Recovery (percent)	Change in Liminal Angle 2nd Exposure (degree)
15	A	35	129	75
	B	55	64	45
	C	60	92	80
	D	60	100	80
16	A	40	88	45
	B	55	91	60
	C	75	107	65
	D	90	100	70
17	A	75	93	55
	B	65	85	45
	C	100	95	70
	D	120	100	95
18	A	55	82	35
	B	55	73	30
	C	85	94	70
	D	70	93	60

Table 3. Petiolar-Response Predictability

Petiole	Plants Fulfilling Criteria 1 and 2	Plants Fulfilling Criteria 1, 2, and 3
D	2	2
	3	3
	4	4
	5	5
	9	9
	15	15
	16	16
	18	18
C	3	3
	4	4
	5	5
	9	-
	10	-
	13	13
	15	15
	17	17
	18	18
B	3	-
	16	16
	17	-
A	1	-
	5	5
	7	7
	12	12
	14	14
	17	17

EXPERIMENTS TO DEFINE THE FLIGHT PACKAGE

Pretested and selected plants were not employed in this series of experiments. It was determined that average data obtained with randomly selected plants would serve adequately as a basis for environmental-condition recommendations.

Temperature Effects

Plants were placed in a controlled-environment chamber for the temperature studies. They were rotated on horizontal clinostats for 24 hours, followed by an additional 24-hour recovery period. Plant responses were compared with the responses of a control group placed inside the chamber and another group maintained at 27 degrees C. The results obtained are summarized in Table 4. The acceptable temperature range appears to be from 21 to 32 degrees C. The reduction in the liminal angle is not significant below 21 degrees C. Temperatures greater than 32 degrees C resulted in plant wilting, although liminal angle response was observed. No attempt was made to stabilize humidity during these tests.

Illumination Effects

Plants were tested for reduction of the liminal angle in total darkness and in light of 2.5 and 10 footcandles. The reduction of the liminal angle was markedly decreased in both tests conducted in the dark. The data obtained are summarized in Table 5. The recovery of the plants following omnilateral geotropic stimulation in the dark was markedly reduced. Greater response with better recovery was found at 2.5 and 10 footcandles. It should be noted that reduction in the liminal angle at the reduced levels of illumination is not as great as those observed under standard illumination (400 footcandles).

Gas Exchange Studies

The nature of the gaseous environment to be provided for the plants within the satellite was considered an important factor, which demanded laboratory investigation. Underlying this investigation was the question of whether or not a circulating gas supply would be required within the plant package. A negative answer to this question would imply simplified package design. It would also result in isolation of the plant package from the satellite's environmental control system, eliminating possible effects due to contaminants arising from other experiments. Potted plants were sealed individually in 4-liter resin reaction vessels. Three of the four plants in the first test grew 15 cm during the first six weeks of confinement. The fourth plant became twisted and distorted after five days. The plants remained green during the entire 90-day test period. At termination of the

Table 4. Reduction of Liminal Angle Versus Temperature

Plant Orientation	Temp (degree C)	Time From Start (hour)	Test Plants ALA (3 Plants) Leaves				Controls** ALA (2 Plants) Leaves			
			A	B	C	D	A	B	C	D
Horizontal	5	0	125	122	135	127	125	122	137	128
Horizontal		24	120	120	132	125	123	120	138	128
Vertical*		48	120	117	132	122	122	123	135	130
Horizontal	10	0	132	135	142	142	112	115	127	130
Horizontal		24	122	120	130	130	112	115	127	132
Vertical*		48	83	85	138	138	112	112	125	127
Horizontal	16	0	140	133	137	135	132	135	122	137
Horizontal		24	110	98	85	75	132	135	132	137
1 g*		48	135	130	128	133	132	130	130	132
Horizontal	21	0	132	117	137	138	125	138	130	138
Horizontal		24	72	74	48	53	130	133	135	135
1 g*		48	127	120	140	135	135	138	135	142

NOTE: ALA = Average Liminal Angle.

Table 4. Reduction of Liminal Angle Versus Temperature (Cont.)

Plant Orientation	Temp (degree C)	Time From Start (hour)	Test Plants ALA (3 Plants) Leaves				Controls** ALA (2 Plants) Leaves			
			A	B	C	D	A	B	C	D
Horizontal	24	0	140	143	140	140	140	130	135	135
Horizontal		24	70	60	75	80	135	130	135	130
1 g*		48	135	127	128	138	135	135	135	135
Horizontal	27	0	127	135	136	135	130	127	147	137
Horizontal		24	62	63	38	42	130	132	143	137
1 g*		48	120	128	133	135	130	130	143	135
Horizontal	29	0	133	127	135	145	135	120	140	150
Horizontal		24	70	55	45	58	130	120	130	145
1 g*		48	115	113	127	143	130	115	130	145
Horizontal	32		135	142	132	138	135	140	138	135
Horizontal		24	42	43	38	38	135	138	140	138
1 g*		48	132	135	132	133	133	140	142	140

NOTE: ALA = Average Liminal Angle.

Table 4. Reduction of Liminal Angle Versus Temperature (Cont.)

Plant Orientation	Temp (degree C)	Time From Start (hour)	Test Plants ALA (3 Plants) Leaves				Controls** ALA (2 Plants) Leaves			
			A	B	C	D	A	B	C	D
Horizontal	35	0	125	138	154	147	115	115	135	130
Horizontal		24	63	55	40	43	105	110	125	115
1 g*		48	93	123	138	140	105	105	125	115
Horizontal	38	0	133	131	142	137	140	133	137	150
Horizontal		24	93	87	90	86	133	133	128	140
1 g*		48	122	122	127	120	130	133	125	135
Horizontal	43	0	138	140	148	152	130	140	160	158
Horizontal		24	WILTED				WILTED			

NOTE: ALA = Average Liminal Angle.
*1 g-loading applied by placing plant upright after removal from clinostat.
**Control plants were placed upright inside the environmental chamber with test plants.

Table 5. Reduction of Liminal Angle Versus Illuminance

Plant Orientation	Illuminance	Time From Start (hour)	Test Plants Average Liminal Angle (3 Plants)				Controls** Average Liminal Angle (2 Plants)			
			A	B	C	D	A	B	C	C
Horizontal	Dark	0	142	137	140	142	135	130	150	150
Horizontal		24	100	90	120	132	140	130	145	150
Vertical*		48	110	100	122	143	135	125	150	155
Horizontal	Dark	0	137	135	135	132	140	140	145	135
Horizontal		24	112	112	90	87	140	135	140	135
Vertical*		48	115	112	95	95	140	135	140	135
Horizontal	2.5fc	0	123	121	138	138	135	125	135	140
Horizontal		24	81	80	65	61	130	125	135	135
Vertical*		48	116	110	138	135	130	125	135	135
Horizontal	10fc	0	137	130	128	140	133	138	130	130
Horizontal		24	88	73	75	83	135	133	130	133
Vertical*		48	130	130	125	143	133	135	130	130

*1 g-Loading applied by placing plant upright following removal from clinostat.
**The control plants were exposed to the lighting conditions but were not rotated on the clinostats.

test, mainstems were quite brittle and could not support the plant body. Leaves were small and delicate. Petioles were elongated (10 to 12 cm). Decreased branching was also noted. The plants began to wilt immediately upon exposure to open air and were dead one day later.

In the second test, three soil-grown plants were sealed in resin reaction vessels for one week. Gas samples were removed from the vessels on a daily basis for oxygen and carbon dioxide analysis. Analyses were performed by absorption methods in a Sholander Apparatus. The data obtained indicated that exchange between the plant and the bacteria present in the soil mixture adequately maintained the atmosphere to permit proper plant growth.

Optimal Plant-Growth Medium

Experiments were conducted to determine the soil medium that would maintain uniform plant growth and retain an acceptable moisture content during flight. Replacement of the usual soil medium with an organic foam support would, if successful, result in a considerable weight saving in the biological package.

The synthetic substrate, Nutri-Foam (Dow Chemical Company), is said to contain the growth factors and nutrients required by plants. It is a spongy, rubber-like material, insect-free and mildew-proof. It is an open-celled foam made from a polyurethane base.

A test was initiated to determine the water-holding capacity of Nutri-Foam. A sample of the medium was cut, measured, and weighed. It was then saturated with water, drained, and reweighed. An 11.5-Gm sample (166 cm^3) retained 73.5-Gm water.

Other experiments were conducted to establish the ability of Nutri-Foam to support plant growth. Plants were grown in water-saturated Nutri-Foam, stored in sealed vessels and on an open shelf, exposed to room air. All six specimens in sealed vessels were observed to defoliate within 24 hours, and all were dead by the third day.

The six specimens cultured open to room air were wilted within 24 hours, and they died within 48 hours. The Nutri-Foam medium of the specimens open to room air was noted to be extremely dry. A second sample of Nutri-Foam was obtained. Its appearance was quite similar to that of the first batch, and was also found to be unsatisfactory for supporting plant growth. A third lot of Nutri-Foam was purchased. It appeared to be grossly different from the previous samples; particles were smaller, within a more uniform and finer-celled foam. Plants were grown in this medium in closed vessels for periods of up to 30 days. Figure 4 is a photograph showing a comparison of the growth in Nutri-Foam (center and left in the photograph) with that in the standard soil mixture (right) under identical conditions. Retarded plant growth and differences in root development were apparent.

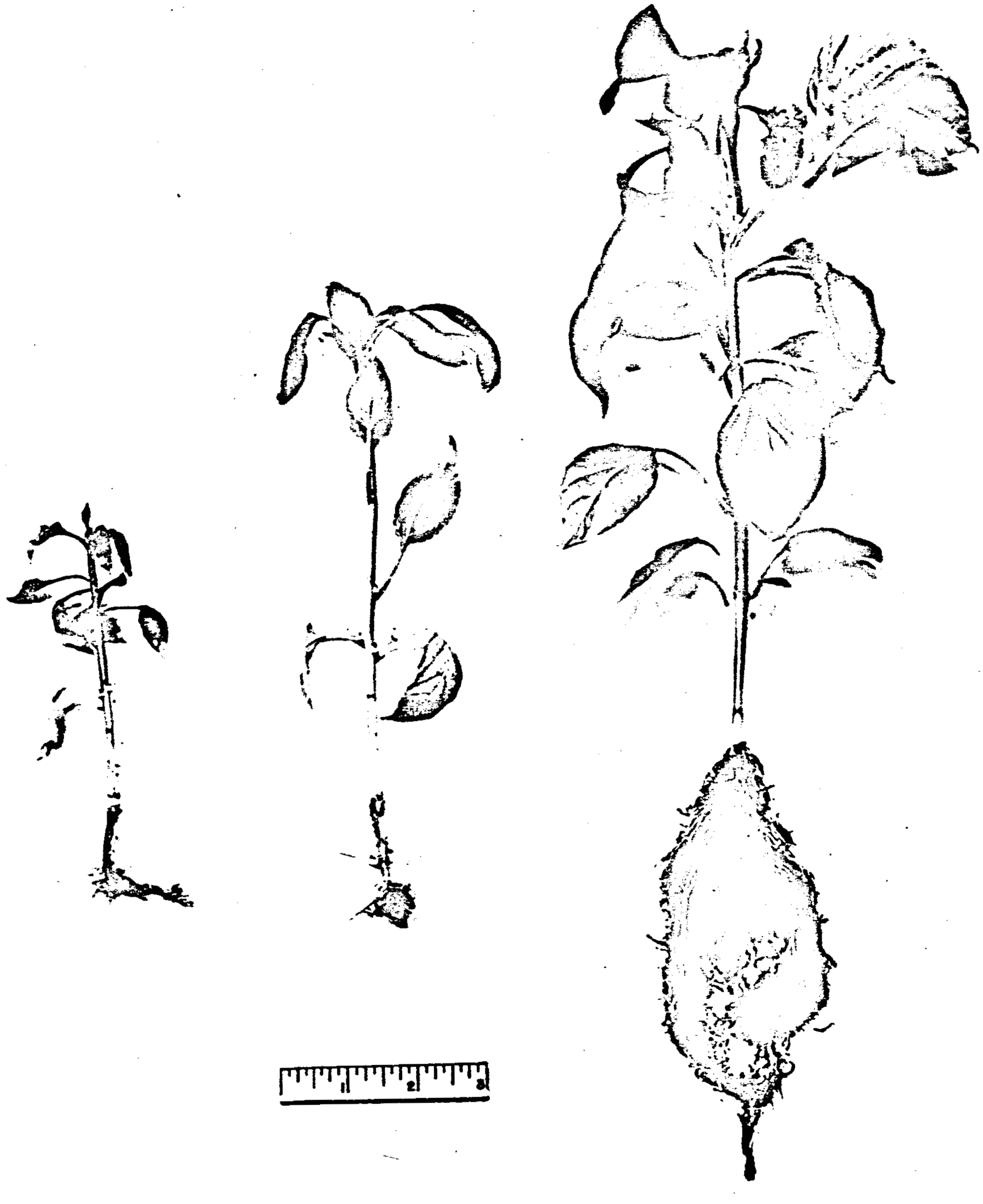

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Figure 4. Comparative Growth in Nutri-Foam and Soil Mixture

Because of these observed results, Nutri-Foam was not used as a substitute for the standard soil mixture in other tests.

Reactions to Stresses

Acceleration and Vibration

Launch vibration and acceleration could degrade plant responses. Plants were placed on clinostats and observed for petiolar response following acceleration and vibration stresses. The following outline was employed as a guide in simulating the predicted vibration and acceleration profile:

1. Vibration in two axes at room temperature

   a. Equipment

      (1) Test fixture to transmit vibration from the exciter to the test package

      (2) An Endevco accelerometer mounted on the test fixture to monitor and control the vibration input levels.

   b. Programming of two sinusoidal sweeps from 10 to 2000 to 10 cycles per second, at a rate of 4 octaves per minute, at the following levels:

	CPS	G s
(1) Thrust Level		
	10 to 50 ±	2.0
	50 to 500 ±	5.0
	500 to 2000 ±	14.0
(2) Lateral Level	10 to 18 ±	2.0
	18 to 500 ±	1.5
	500 to 2000 ±	2.5

2. Acceleration exposure in one axis at room temperature

   a. Equipment

      (1) Test package mounted on a test fixture

      (2) Rotary accelerator

   b. Program: Linear acceleration for 7 minutes to simulate the launch-to-orbit profile of the Biosatellite spacecraft. (Note: Accelerations were approximated within the limitations of the existing NAA equipment.) Three plants were individually packaged in plastic containers of soil and subjected to the dynamic vibration and acceleration tests. The liminal angles of each plant were then recorded. The plants were subsequently subjected to 24 hours of horizontal clinostat rotation. Response of the petioles was again recorded. Recovery of each petiole was also measured.

No significant changes in the liminal angle resulted from exposure to the dynamic test profile. The plants then responded to horizontal rotation in the usual manner, and recovered as expected. The data indicated that *Capsicum annuum* is capable of withstanding the anticipated biosatellite launch profile without impaired petiolar response.

Long-term horizontal rotation

Eight plants were rotated on horizontal clinostats for 134 hours. Petioles A and B showed an average 40-degree reduction in liminal angle. Petioles C and D showed an average 90-degree reduction in liminal angle. The plants were subsequently allowed a 24-hour recovery period. All petioles recovered, but petioles C and D showed a greater percentage of recovery.

One plant was rotated on a horizontal clinostat for 30 days. The plant exhibited abnormal form upon removal from the clinostat. The plant did not recover completely. Two plants were rotated for 60 days. Both exhibited gross anatomical abnormalities. Both plants exhibited chlorosis after approximately 50 days and died within one week after removal from the clinostats. Plants were maintained on the regular nutrient feeding schedule during all long-term rotation tests. No buds were observed on the plants during the 60-day rotational exposure.

Exposure to Super-g

Eight plants were exposed to 4.6 g on the centrifuge-clinostat device for 24 hours. The liminal angles were greatly reduced, probably due to the great centrifugal forces imparted by this exposure. The plants were removed from the centrifuge and allowed a 24-hour recovery period. The petioles failed to recover more than a few degrees.

Effects of Leaf Trimming

Tests were undertaken to determine the maximum amount of trimming of plant parts which would not impair leaf response to facilitate photographic coverage. Initial testing, on a 24-hour basis, has shown that the apical bud and all leaves, except for the few to be studied, may be removed without disturbing normal reaction to horizontal rotation.

In other tests, petioles A, B, C, and D were debladed to observe the response of the petiole. Five plants were exposed to 24 hours of horizontal rotation in this condition. The petioles responded in all cases, but to a lesser extent than did bladed petioles. In addition, poor recovery was noted 24 hours after the plants were removed from the clinostats.

Exposure to Reduced Pressure

Three plants were placed on horizontal clinostats in a controlled-environment chamber, maintained at 5-psia air under 10-footcandle illumination for 24 hours. Poor petiolar response was noted in all cases. Recovery was permitted under the same environmental conditions, and all petioles recovered well.

LABORATORY MODEL

Determination of the Number of Plants

Initial limitations in the number of plants to be used in the scientific package resulted from weight and volume restrictions. Determination of a three-plant package was based upon statistical analysis of test data. Responses of plants on clinostats were tested using a "randomized-block" analysis of variance. These tests indicated that significant treatment effects could be obtained with samples as small as three plants, provided these are preselected for uniform response. Preselected plants would also make it possible to obtain valid reference points for comparison with in-flight measurements, further increasing the significance of the data obtained. It was also concluded that the added statistical value of a fourth plant was not sufficient to warrant the increased weight, volume, and complexity in packaging and photographic coverage.

Design

Design of the laboratory model was based on use of a minimum of three plants, 3 inches high and 3-1/2 to 4 inches in diameter-an additional inch in height provided for growth. The mission duration was considered to be seven days or more, requiring pots 2-1/2 inches in diameter and 3 inches high to contain root medium (soil) and moisture, and requiring a minimum light source of 10 footcandles. The design includes mirrors to facilitate camera coverage. The plants are housed in individual pots, with covers to prevent the loss of soil and to minimize evaporative losses. A vertical rod was provided for each plant to support the stem axis. A shield was placed on three sides of the package to reduce the effect of air currents on the position of the petioles.

(Refer to Appendix C, for representations of the preliminary design for the laboratory model, based on the preliminary specifications listed above.)

Provision was made for photographic coverage; however, a camera was not incorporated into the package design. In order to obtain photographic coverage of the three plants, it was assumed that plants would be selected which had two petioles each in two vertical planes approximately perpendicular to each other. The petioles in one plane would be placed normal to the line of sight from the camera lens. A mirror would reflect a picture normal to the petioles in the other plane. The mirrors were marked off in a grid of 1-cm squares in order to permit corrections for any distortion due to view angle. The pots and mirrors were located so as to eliminate interferences with the lines of sight from the camera lens.

Fabrication

The laboratory model based on the design drawings (Figures C-1 through C-3) was constructed by the Los Angeles Division, North American Aviation, Inc. Except for the mirrors and shockmounts, the unit was fabricated of weldable sheet aluminum. The completed unit is illustrated in Figure 5.

Stress Tests

Vibration and acceleration tests were undertaken to determine the interactions between the package and the biological specimens caused by exposure to a simulated launch profile. The results would serve as guidelines in the design of future hardware.

Vibration and acceleration profiles identical to those specified on page 23 were employed.

The laboratory model demonstrated the capability of the plants and hardware to withstand the dynamic forces of a typical Biosatellite flight profile.

Interfaces

Although hardware development has not progressed beyond the preliminary stage, it nevertheless has been important to consider interrelationships between the experimental package and other components of the flight system. These interfaces are discussed below:

1. Power - 5 watts, 28 volts D-C average, continuous, to power the camera and associated lighting, assuming photographic lighting is sufficient to permit adequate plant response.

2. Environmental Control System

   a. Atmosphere - cabin maintained at 15 psi with oxygen partial

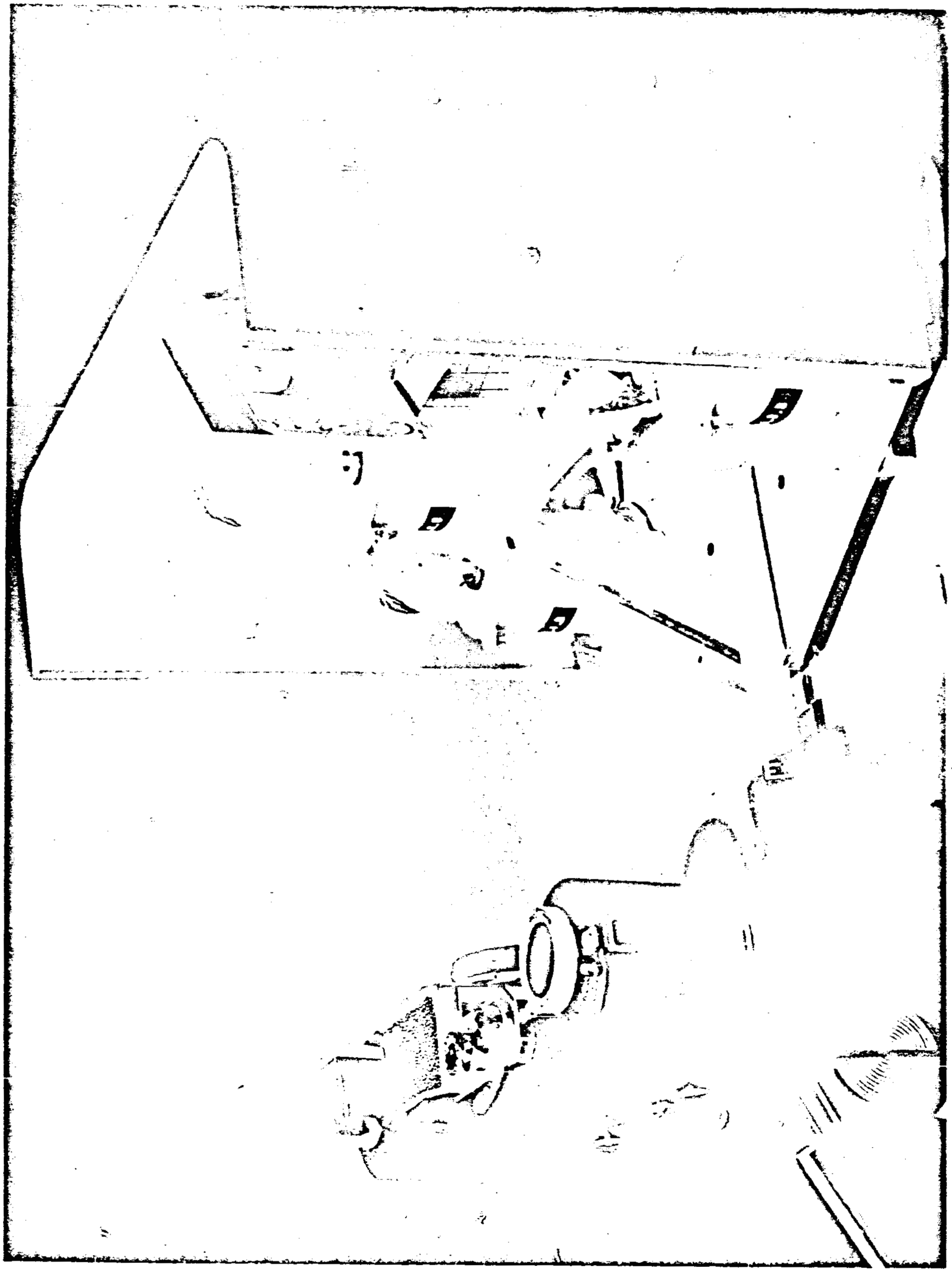

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Figure 5. Laboratory Model

pressure not less than 156-mm Hg; carbon dioxide not less than 0.23-mm Hg.

b. Temperature - 27± 2 degrees C preferred.

c. Humidity - 40-75 percent rh

d. Contaminants - minimal interference from other experiments and capsule hardware.

3. Command - none required for biological package. Camera requires signals during specific mission phases.

4. Sensors - Thermistors in the vicinity of each plant. Data storage for thermistor output.

5. Spatial and Weight - Package is approximately rectangular in shape, which will include a camera, 21-cm high, 24.0-cm wide, and 30.0-cm long. Volume 15,000 cm^3 (approximately) and weight 5-350 grams (approximately).

Delivery to Customer

The laboratory model which was delivered to Ames Research Center on 8 January 1964 was accepted. NASA and NAA personnel discussed modifications, spatial arrangement of the plants for better photographic coverage and treatment of the plants (e.g., deleafing).

SUMMARY DISCUSSION AND RECOMMENDATIONS

The plant species, Capsicum annuum (Yolo Wonder Bell Pepper), was selected as the test subject for use in the experimental program. In addition to its potential as a crop plant, its small size, durability, and petiolar responsiveness made it the plant of choice.

The most significant finding in this phase of the program was the strong indication that predictably responsive plants could be selected prior to flight. Refined measurement techniques, involving both mechanical and photographic methods, can not be expected to increase the reliability of the experiment unless residual variability among plant specimens is minimized.

It is realized that selection procedures would not permit the observance of effect upon a representative sample of the population. Selection would, however, assure that the three plants selected for flight will maximize the probability of uniform behavior. It is recommended that further investigation of the selective process be emphasized in future studies. Verification of plant selection by pretesting would necessitate repetition of many previous tests, utilizing selected plants. The immediate goal of future studies should be the production and selection of plants in which all petioles respond in a predictable manner.

Studies to define many of the parameters important to the design of the laboratory model were reported in the body of the report.

Studies indicated that a temperature range of 21 to 32 degrees C is satisfactory for plant response. Further studies are required to differentiate temperature effects from humidity effects. Plagiogeotropic response in total darkness was considerably lower than that observed in plants cultured under constant illumination. It is realized that a high power requirement for constant illumination is a severe disadvantage. Studies to investigate petiolar response under the intermittent illumination required for photographic coverage are therefore recommended. Experiments performed in this phase of the study indicated that a closed package could be utilized. It was decided to design an open package to permit adaptability of the experiment to various mission durations. In the event that a closed package is ultimately designed, two additional factors should be considered. First, the symbiotic effects with soil and plant bacteria should be determined. Second, the plagiogeotropic response of plants in closed vessels should be studied.

The most satisfactory culture medium employed during the studies was a soil mixture to which a nutrient supplement was added. The synthetic substrate, Nutri-Foam, was evaluated and found to be unsatisfactory. Further investigations are recommended to investigate the use of other substrates to optimize plant growth and response.

Tests todetermine the plant's ability to withstand expected acceleration and vibration indicated that petiolar response was not significantly affected. Capsicum, therefore, may be expected to endure the dynamic stresses of space flight without losing its ability to elicit plagiogeotropic response.

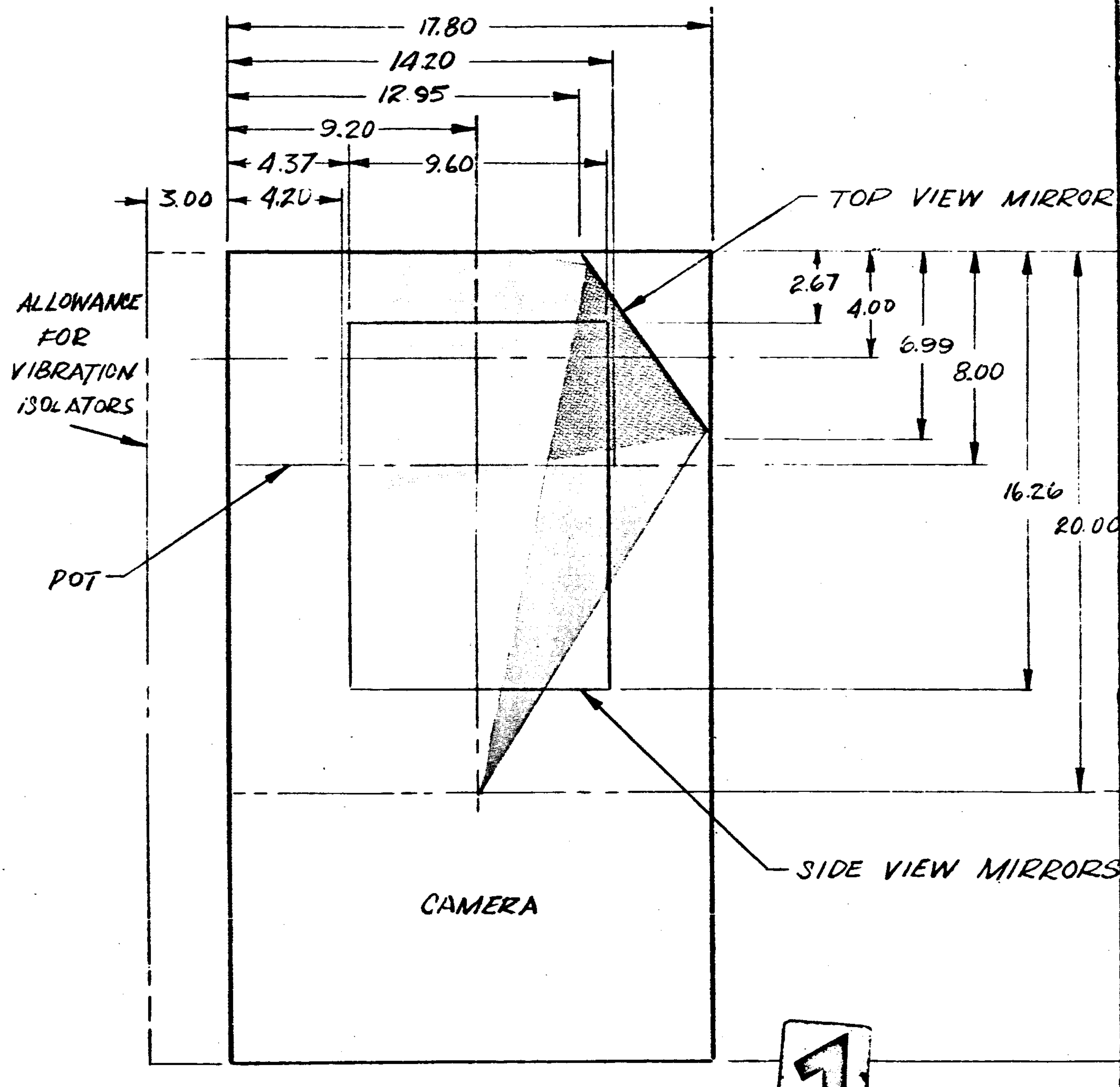
The recovery of pepper plants after prolonged periods of horizontal rotation may provide an indication of plant response and growth in the reduced gravitational fields to be expected in space installations. Such studies would indicate whether corrective measures (viz., centrifugation) might be required to counteract adverse effects of chronic exposure to weightlessness. Studies to determine the effects of leaf trimming were performed to limit interference in photographic interpretation and to demonstrate the response of debladed petioles. Preliminary predictive data demonstrated that petioles C and D were more reliable indicators of petiolar response. If further studies show that only petioles C and D are valid for predictive purposes, studies should be performed to determine the ability of a two-petiole plant (i.e., young petioles) in this investigation.

Tests performed by NAA in which plants were exposed to air at reduced pressure (viz., 5 psia) indicated that petiolar response remained satisfactory. In the event that plant growth in a reduced pressure is required because of Biosatellite capsule constraints or future space-station applications, an extension of these studies to include various gas compositions is recommended.

The use of three plants in the laboratory model was supported by statistical analysis. Validation of the pretest selection technique will further enhance statistical reliability. Figure 6 illustrates a proposed modification of the original laboratory model. In this design, all plants are on one level to compress the package. Further design investigations are recommended to result in additional savings in weight and volume. Baffles should be designed, if necessary, to prevent air currents within the vehicle from affecting any response of the test specimens in the zero-gravity environment. Methods to provide quick interchangeability and access to the enclosed plants should also be investigated.

Photographic coverage during flight should be carefully planned and executed during design phases. Photographic equipment provided for in-flight monitoring of the biological package should fulfill the following requirements:

1. The camera should be programmed by its own internal mechanism to take motion pictures for approximately 4 minutes during launch, then



NOTE - DIMENSIONS IN CENTIMETERS

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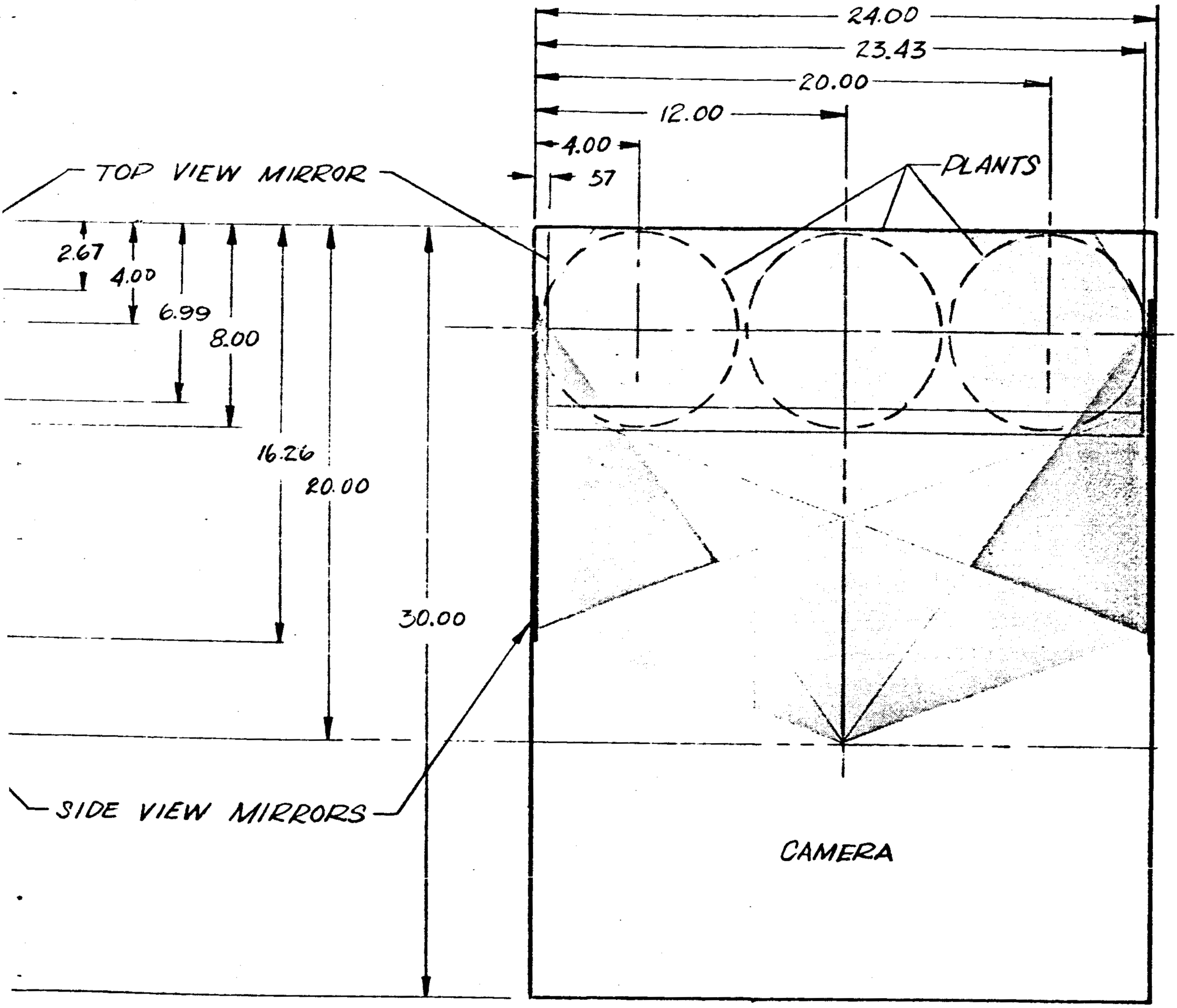

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Figure 6. Arrangement of Plants, Mirrors, and Camera for Liminal Angle Experiment

one frame every 10 minutes for approximately 72 hours during orbit, and, finally, another 4 minutes of motion pictures upon reëntry of the capsule.

2. The camera chould have its own light source and electric drive.

3. The camera should not weigh more than 1361 grams at launch.

4. By strategic location of mirrors and orientation of plant petioles, coverage in three planes should be available in the photographs.

One plan for the location of equipment to facilitate adequate photographic coverage of all plants is revealed in Figure 6.

Expected interfaces with the Biosatellite capsule were enumerated. It appears that the biological package will be amenable to the Biosatellite capsule's environment.

APPENDIX A: COMPOSITION OF NUTRIENT SOLUTION

Stock Solution A (Macronutrients)

	g/L Distilled Water
$NH_4H_2PO_4$	4.0
$MgSO_4 \cdot 7H_2O$	0.5
$Ca(NO_3)_2 \cdot 4H_2O$	1.0
KCl	1.0

Stock Solution B (Micronutrients) Modified Arnon's A-5 Stock Solution

	g/L 0.01 N H_2SO_4
H_3BO_3	2.86
$MnCl_2 \cdot 4H_2O$	1.81
$ZnSO_4$	0.22
$CuSO_4 \cdot 5H_2O$	0.079
$(NH_4)6Mo_7O_{24} \cdot 4H_2O$	0.0177
$Na_3VO_4 \cdot 16H_2O$	0.042

Stock Solution C (Fe++)

Dissolve 20 g of EDTA in one liter of distilled H_2O. Adjust pH to 3.5 with dilute HCl (1.2N). Add 10 g of $FeSO_4 \cdot 7H_2O$. Adjust pH to 7.5-8.0 with dilute NaOH (1N). Approximate final concentration: 2g Fe++/L. To make solution:

1. Dilute stock solution A by adding 9 parts of distilled water.
2. Add 12 ml of stock solution B/liter of diluted stock solution A.
3. Add 2 ml of stock solution C/liter of diluted stock solution A.

APPENDIX B: PLANT RESPONSE

Table B-1. Plant Response at Two and Three Weeks

Plants	Condition	Time From Start (hours)	Average Liminal Angle (degree)			
			A	B	C	D
TWO WEEKS						
1 to 8	Horizontal	0	151	146	180	180
	Horizontal	24	151	140	180	180
	1 g *	48	150	148	180	180
9 to 16	Controls	0	159	156	178	180
		24	155	160	178	180
		48	154	155	180	180
17 to 24	Horizontal	0	152	151	179	179
	Horizontal	24	134	145	180	180
	1 g *	48	147	150	180	180

*g-loading applied by placing clinostats upright.
**g-loading applied by centrifugation.

Table B-1. Plant Response at Two and Three Weeks (Cont.)

Plants	Condition	Time From Start (hours)	Average Liminal Angle (degree)			
			A	B	C	D
THREE WEEKS						
1 to 8	Horizontal	0	137	137	160	166
	Horizontal	24	117	118	133	132
	1 g *	48	139	140	154	160
9 to 16	Horizontal	0	141	145	156	160
	Horizontal	24	142	140	149	153
	Horizontal	48	142	140	149	153
17 to 24	Horizontal	0	130	152	160	161
	Horizontal	24	101	136	140	133
	1 g *	48	130	139	143	137

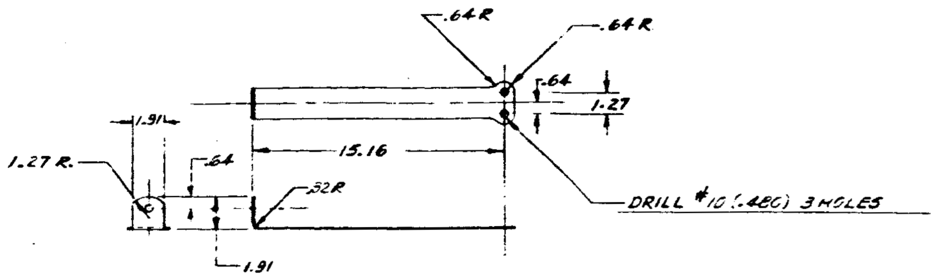
*g-loading applied by placing clinostats upright.
**g-loading applied by centrifugation.

Table B-2. Plant Response at Four Weeks

Plants	Condition	Time From Start (Hours)	Average Liminal Angle (degree)			
			A	B	C	D
FOUR WEEKS						
1 to 8	Horizontal	0	122	123	145	158
	Horizontal	24	44	42	100	121
	1 g *	48	83	83	140	150
9 to 16	Horizontal	0	120	126	168	165
	Horizontal	24	29	27	144	149
	1 g **	48	105	103	164	165
17 to 24	Controls	0	131	132	151	144
		24	129	130	149	140
		48	128	130	147	142

*g-loading applied by placing clinostats upright.
**g-loading applied by centrifugation.

APPENDIX C: PRELIMINARY DESIGN OF LABORATORY MODEL



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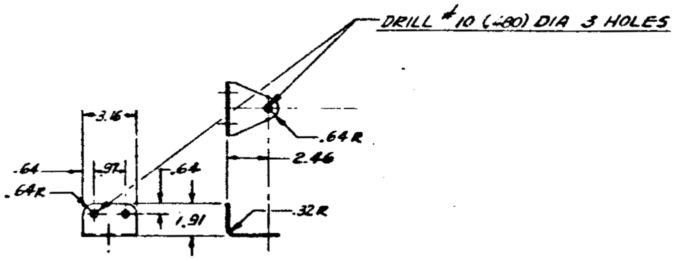

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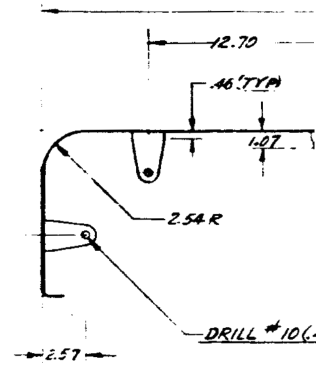

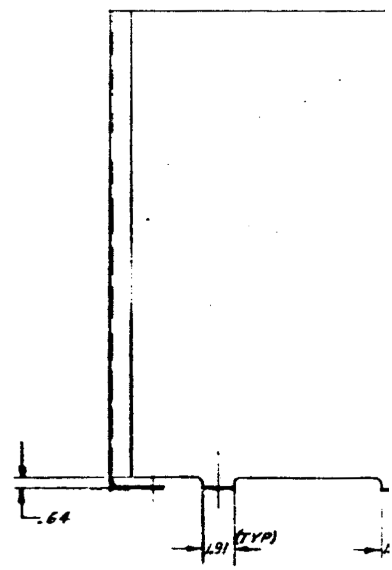

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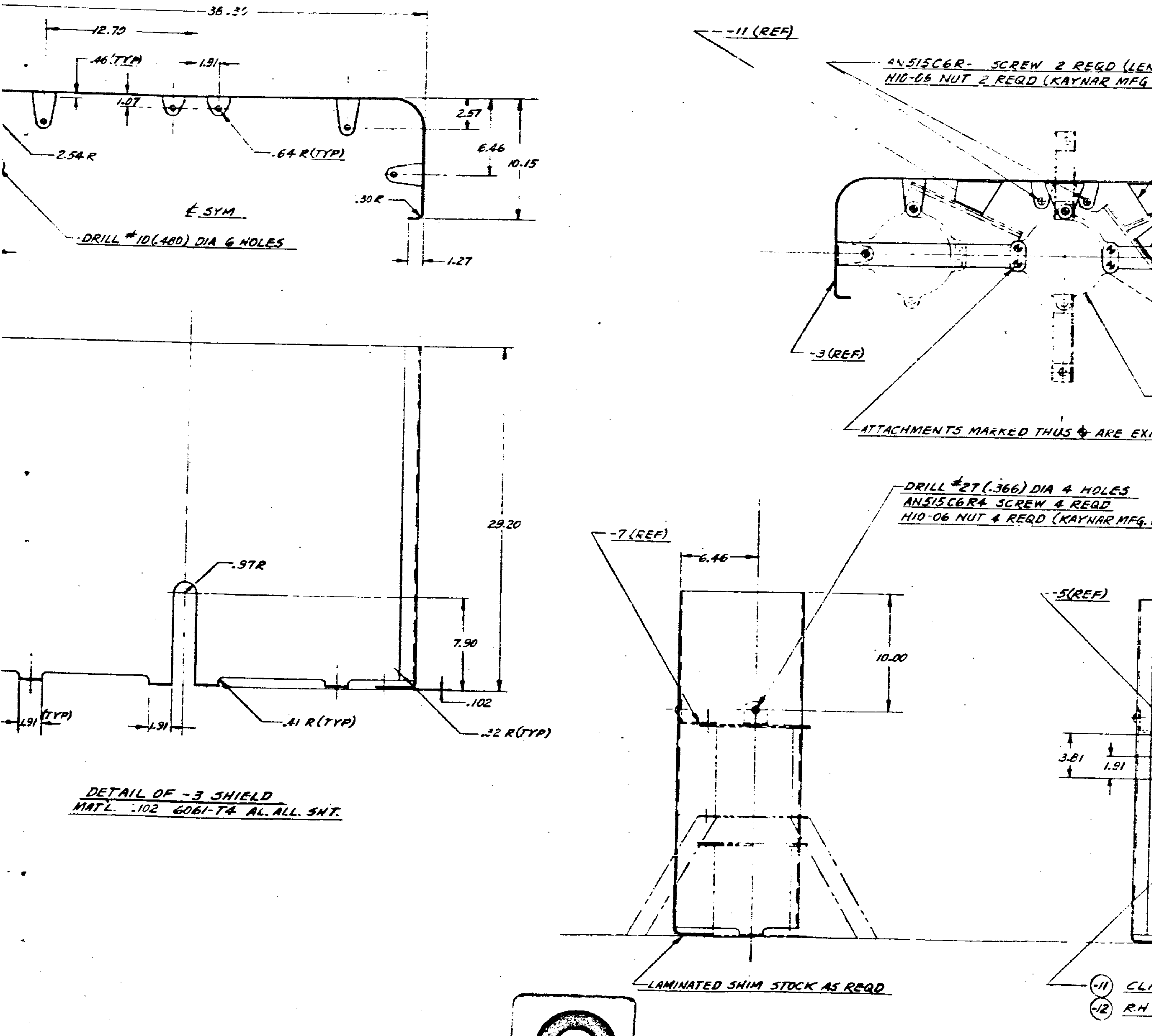
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Figure

2

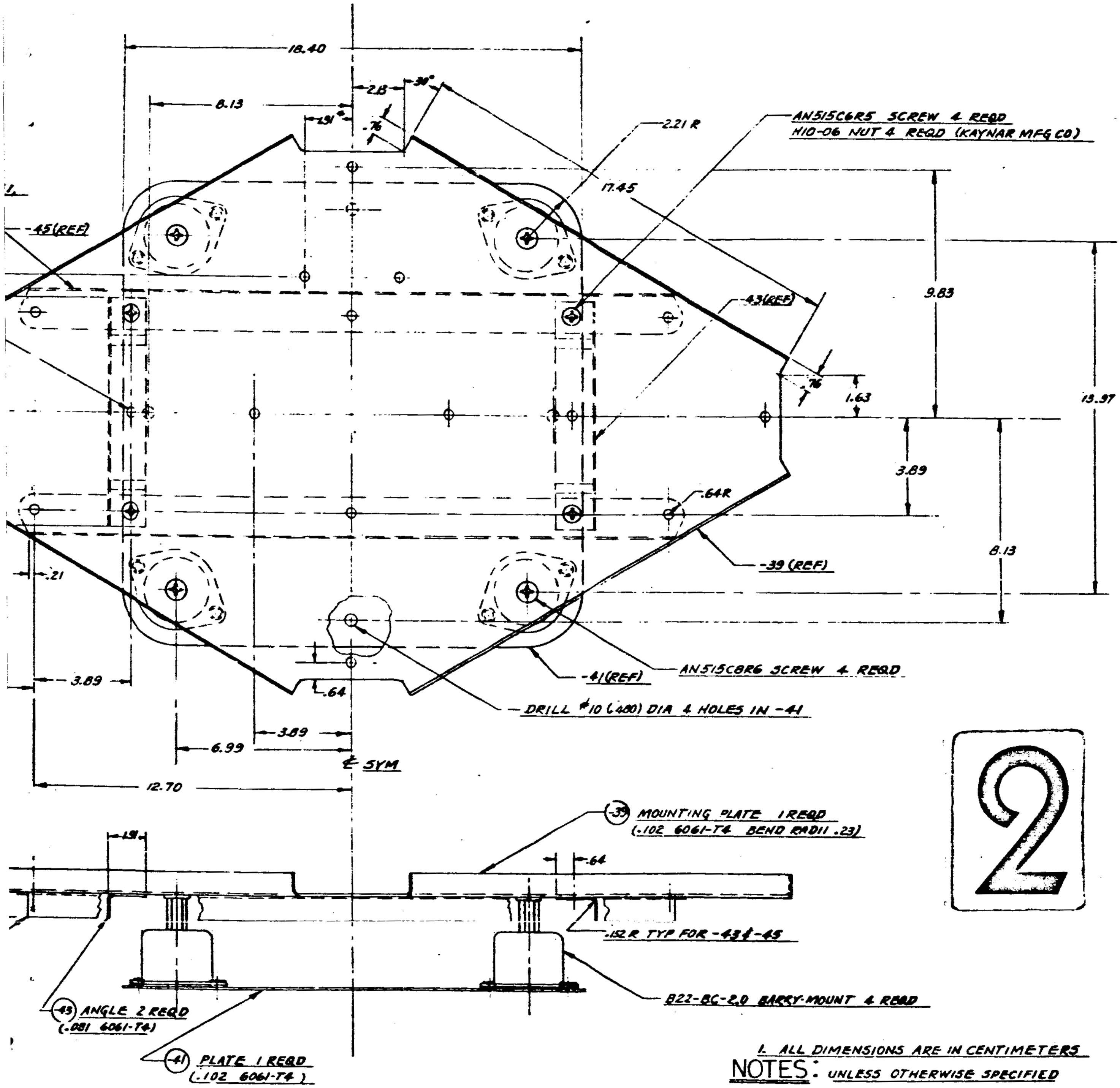

Figure C-3. Container Arrangement - Zero-Gravity Plant Growth Experiment

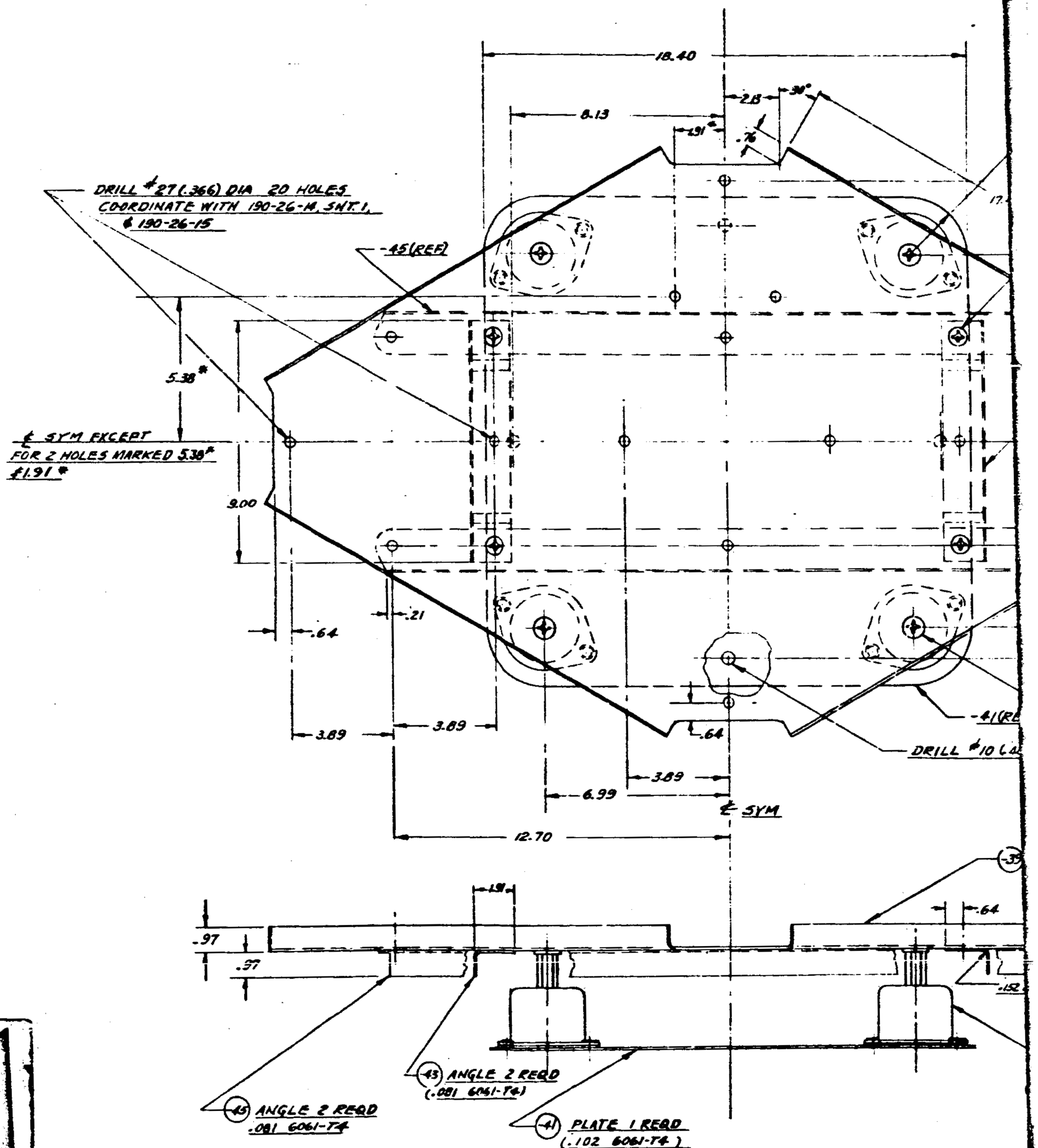

Figure C-3. Container Arrangem

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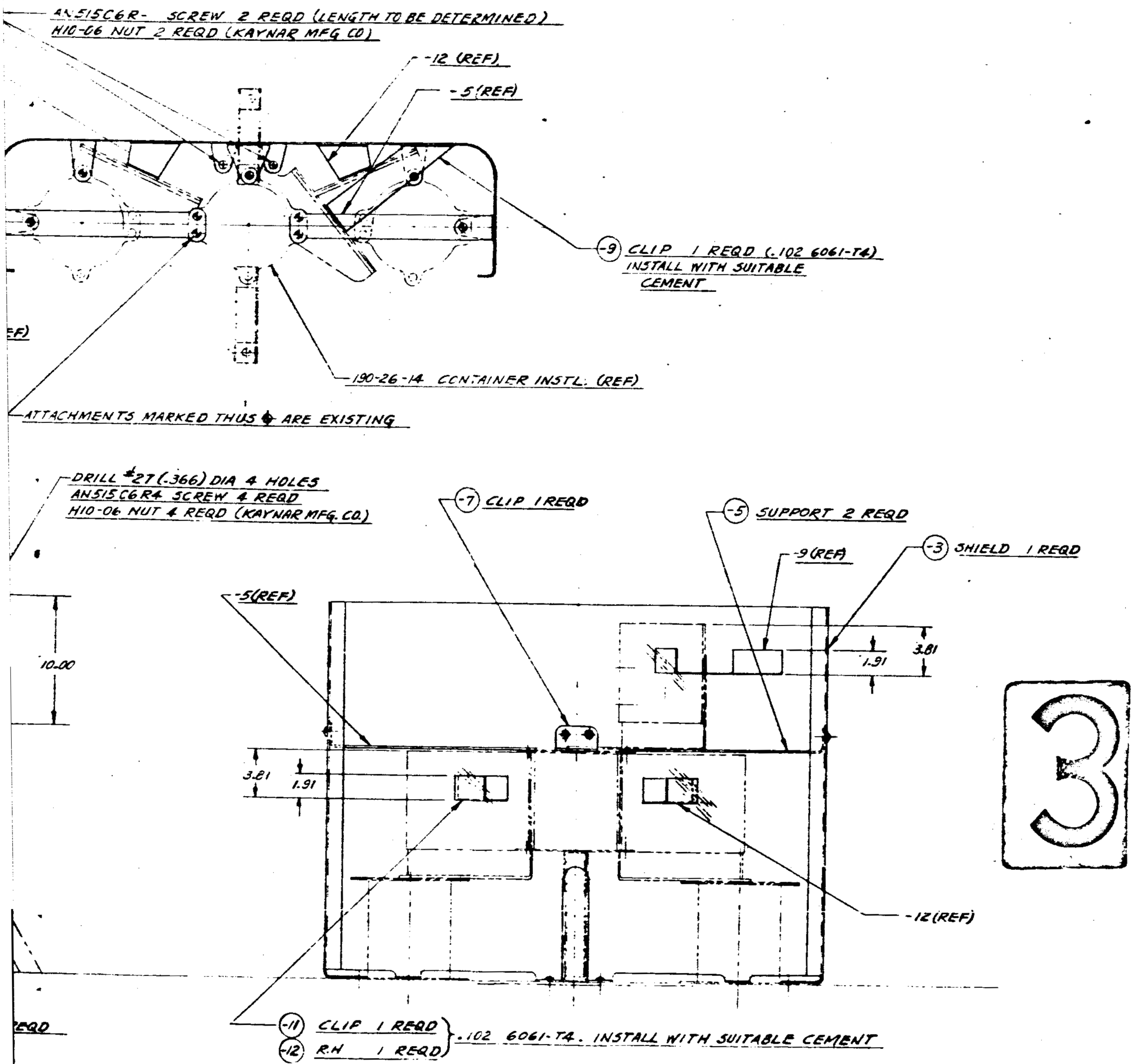

Figure C-1. Shield Installation - Zero-Gravity Plant Growth Experiment

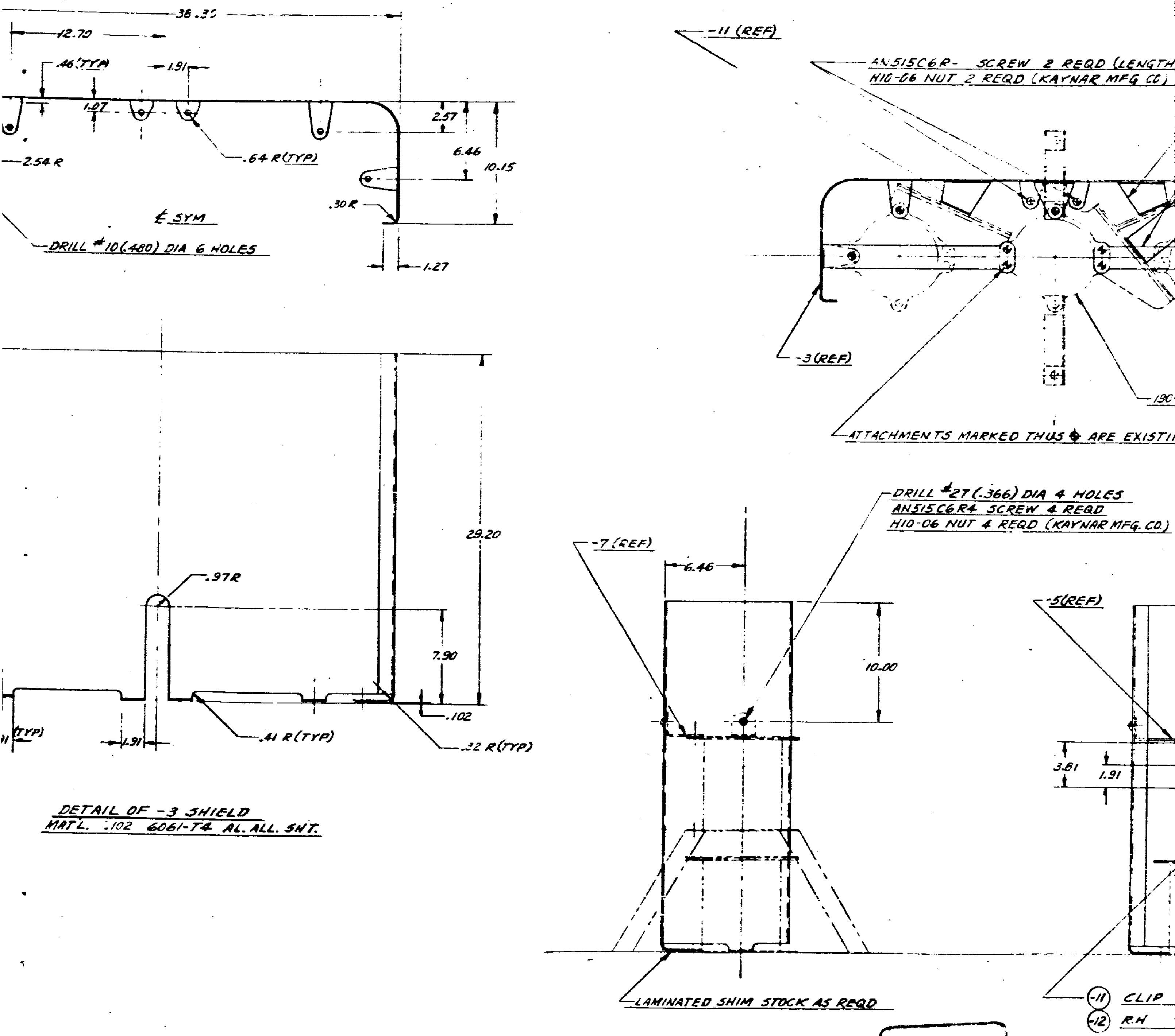
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Figure C-

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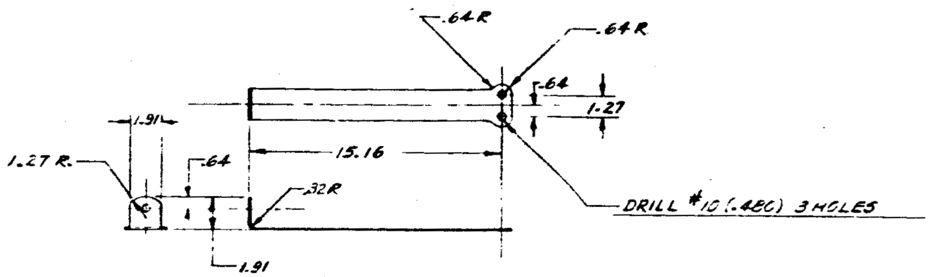

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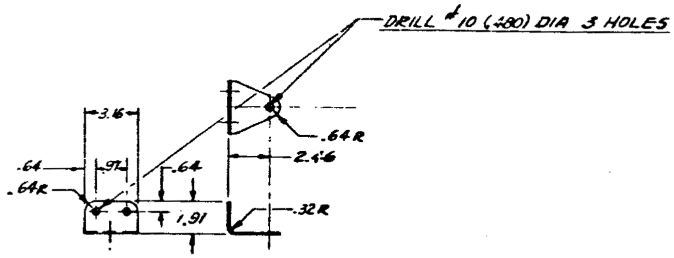

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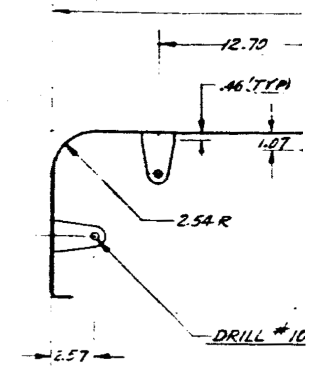

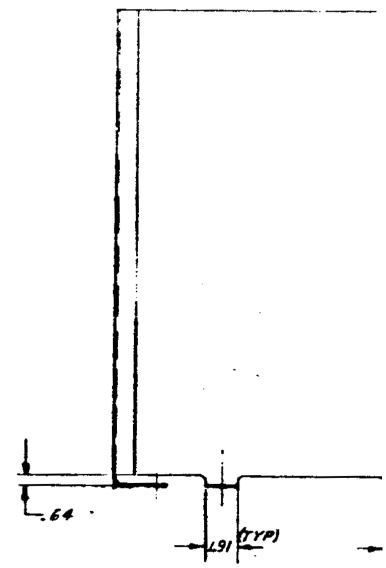

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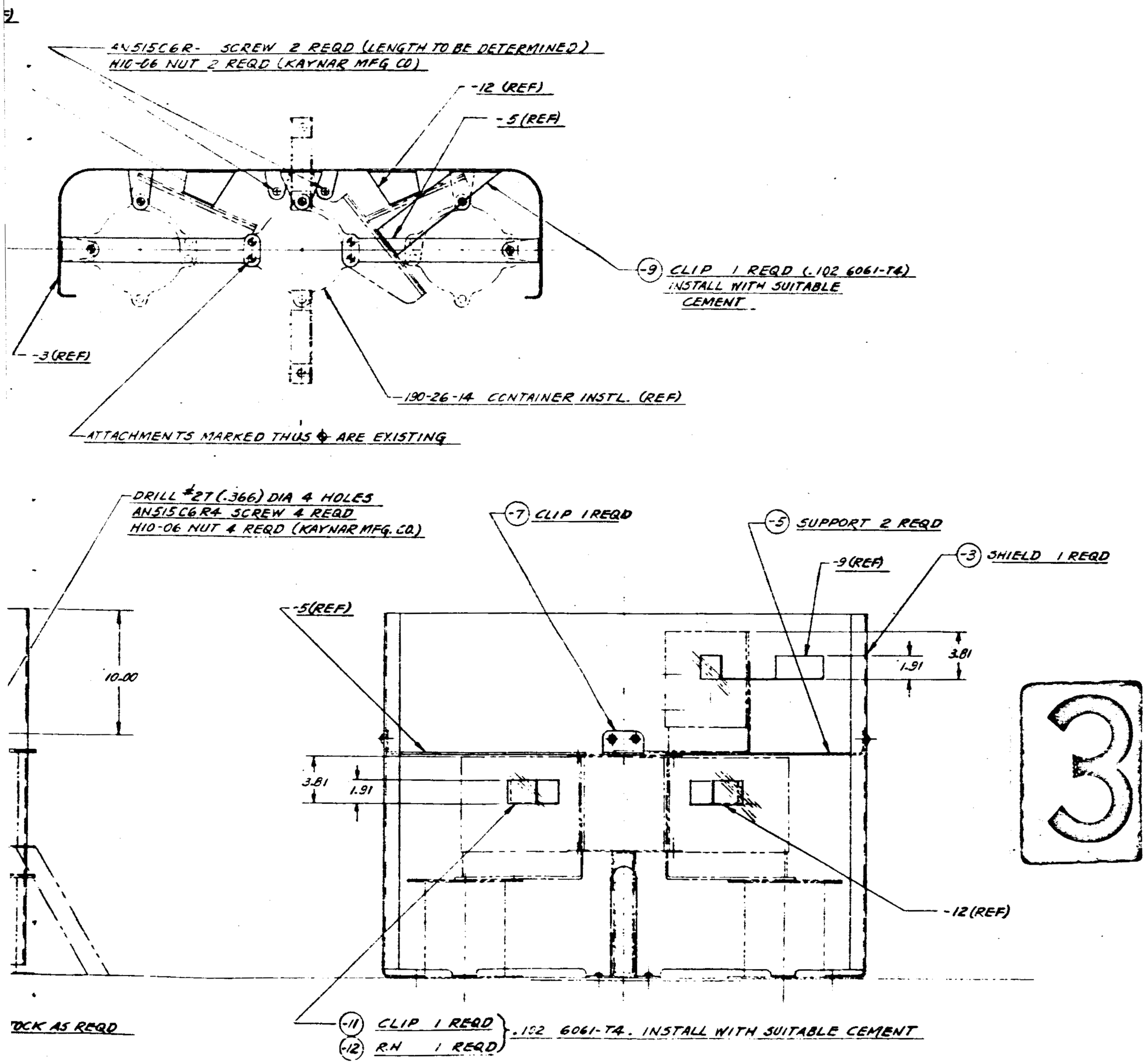

Figure C-1. Shield Installation - Zero-Gravity Plant Growth Experiment